CLAIMS

1. An engineered cytokine-induced memory-like human NK cell or a population of said cells, wherein the engineered NK cell or population of said cells expresses a chimeric antigen receptor (CAR) polypeptide comprising an intracellular domain, a transmembrane domain and an extracellular binding domain, wherein the extracellular binding domain specifically binds to an antigen comprising an NPM1c neoepitope in complex with a class I major histocompatibility complex (MHC class I) protein.

2. The engineered NK cell or population of cells of claim 1, wherein the extracellular binding domain does not bind to, or substantially does not bind to: (a) the MHC class I protein alone, and/or (b) a control peptide in complex with the MHC class I protein, optionally wherein the control peptide is an NY-ESO-1 epitope or influenza virus M1 epitope.

3. The engineered NK cell or population of cells of claim 1 or 2, wherein the NPM1c neoepitope comprises an amino acid sequence $X_1X_2X_3X_4X_5X_6X_7X_8X_9$, wherein X_1 is selected from A, V, L or I, wherein X_2 is selected from A, T, S, V, L, I, M or Q, wherein X_3 is selected from Q or N, wherein X_4 is selected from D or E, wherein X_5 is selected from L, I, V, M, A or F, wherein X_6 is selected from C, S, or A, wherein X_7 is selected from L, I, V, M, A, or F, wherein X_8 is selected from A, V, L or I, and wherein X_9 is selected from L, I, V, M or A.

4. The engineered NK cell or population of cells of any one of claims 1-3, wherein the NPM1c neoepitope comprises an amino acid sequence selected from: AIQDLCLAV (SEQ ID NO:1) or AIQDLCVAV (SEQ ID NO: 71).

5. The engineered NK cell or population of cells of claim 1 or 2, wherein the NPM1c neoepitope comprises an amino acid sequence selected from: CLAVEEVSL (SEQ ID NO:72), VEEVSLRK (SEQ ID NO:73), AVEEVSLR (SEQ ID NO:74), AVEEVSLRK (SEQ ID NO:75), CLAVEEVSLRK (SEQ ID NO:76).

6. The engineered NK cell or population of cells of any one of claims 1-5, wherein the extracellular domain comprises:

(i) a heavy chain variable region (VH) comprising VH complementarity determining region (CDR)1, VH CDR2 and VH CDR3, said VH CDR1, VH CDR2 and VH CDR3 being the CDRs of a VH that has an amino acid sequence of SEQ ID NO: 5, and/or

(ii) a light chain variable region (VL) comprising VL complementarity determining region (CDR)1, VL CDR2 and VL CDR3, said VL CDR1, VL CDR2 and VL CDR3 being the CDRs of a VL that has an amino acid sequence of SEQ ID NO: 3.

7. The engineered NK cell or population of cells of claim 1 or 2, wherein the extracellular domain comprises a scFV, wherein the scFV comprises a Gly-Ser linker selected from the group consisting of (Gly4Ser) (SEQ ID NO:58), (Gly4Ser)2 (SEQ ID NO:59), (Gly4Ser)3 (SEQ ID NO:60), and (Gly4Ser)4 (SEQ ID NO:61).

8. The engineered NK cell or population of cells, according to any one of claims 1-7, wherein the antigen is on the surface of a cancer cell.

9. The engineered NK cell or population of cells of any one of claims 1-8, wherein the transmembrane domain comprises the transmembrane domain of CD3-zeta, CD8, CD28, DAP12, 2B4, NKG2D, CD16, NKp44 or NKp46.

10. The engineered NK cell or population of cells of any one of claims 1-9, wherein the intracellular domain comprises one or more costimulatory domains of one or more costimulatory molecules selected from the group consisting of: CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICOS, 2B4, DAP10, CD137 and DAP12.

11. The engineered NK cell or population of cells of any one of the claims 1-10, wherein the intracellular domain further comprises a self-cleaving peptide sequence and a cytokine, wherein cleavage of the self-cleaving peptide releases the cytokine, wherein the cytokine is IL-12, IL-7, IL-13, IL-15, TNF-α, IFN-γ, or CCL19.

12. The engineered NK cell or population of cells of any one of claims 1-11, which

(i) produces increased IFNγ in the presence of one or more cytokines and/or tumor targets relative to a control human NK cell or human NK cell line;

(ii) has enhanced antibody-dependent cellular cytotoxicity relative to a control human NK cell or human NK cell line; and/or

(iii) has enhanced anti-tumor efficacy relative to a control human NK cell or human NK cell line,

optionally wherein the control NK cell is a human NK cell activated in the presence of IL-15 alone, or a human NK cell line activated in the presence of IL-15 alone.

13. The engineered NK cell or population of cells of any one of claims 1-12, which expresses an IL-15 polypeptide, optionally a human IL-15 polypeptide,
wherein the IL-15 polypeptide is a secreted IL-15 polypeptide or a membrane bound IL-15 polypeptide.

14. The engineered NK cell or population of said cells of any one of claims 1-13, wherein the engineered NK cell or population of said cells contacted with a target cell presenting the NPM1c neoepitope in complex with a MHC class I protein

(i) has increased expression of IFN gamma relative to a control population of NK cells;

(ii) has increased expression of granzyme B relative to a control population of NK cells;

(iii) has increased expression of one or more activation markers relative to a control population of NK cells, wherein the one or more activation markers are selected from: CD25, CD69, ICOS, CD226, CD107a, and CD62L;

(iv) has increased expression of one or more activating receptors relative to a control population of NK cells, wherein the one or more activating receptors are selected from: NKp30, NKG2D, NKp44;

(v) has increased expression of one or more maturation markers relative to a control population of NK cells, wherein the one or more maturation markers are selected from: CD56 and NKG2A;

(vi) has decreased expression of CD57 relative to a control population of NK cells;

(vii) has increased expression of TIGIT relative to a control population of NK cells; and/or

(viii) has decreased expression of TRAIL relative to a control population of NK cells; optionally wherein the control population of NK cells is an untransduced population of cytokine-induced ML NK cells.

15. A pharmaceutical composition, comprising the engineered cell or population of cells of any one of claims 1-14, and a pharmaceutically acceptable carrier.

16. A method for producing the engineered cell or population of cells of any one of claims 1-15, wherein the method comprises:

(i) obtaining a primary human NK cell or population of said cells;

(ii) contacting the NK cell or population of said cells with an amount of IL-12, IL-15, IL-18, or any combination thereof, for a period of time sufficient to obtain a cytokine-induced memory-like NK cell or population of said cells;

(iii) contacting the NK cell or population of said cells of step (ii) with a lentiviral vector encoding the CAR polypeptide under conditions to transduce the NK cell or population of said cells;

(iv) isolating the NK cell expressing the CAR polypeptide or population of said cells; and

(v) optionally, expanding the isolated cell.

17. The method of claim 16, wherein the primary human NK cell or population of said cells is derived from an iPSC, cord blood, or PBMCs.

18. A method of producing a population of engineered cytokine-induced memory-like (ML) NK cell expressing a heterologous polypeptide, the method comprising:

contacting a population of cytokine-induced ML NK cells expressing ASCT-2 with a pseudotyped lentiviral vector encoding the heterologous polypeptide under conditions to transduce the population,

wherein the pseudotyped lentiviral vector comprises a glycoprotein that binds to ASCT-2,

thereby producing the population of engineered cytokine-induced ML NK cells expressing the heterologous polypeptide.

19. The method of claim 18, wherein the cytokine-induced ML NK cells are obtained from a population of primary human NK cells.

20. The method of claim 18 or 19, wherein the population of primary human NK cells is derived from iPSCs, cord blood, or PBMCs.

21. The method of any one of claims 18-20, wherein the population of primary human NK cells is autologous or allogeneic.

22. The method of any one of claims 18-21, wherein expression of ASCT-2 is increased in the population of cytokine-induced ML NK cells relative to a control population of human NK cells by about 1.3-fold, 1.4-fold, 1.5-fold, 1.6-fold, 1.7-fold, 1.8-fold, 2-fold, 2.5-fold, 3-fold, 3.5-fold, or 4-fold, optionally wherein the control population of human NK cells is activated in the presence of IL-15 alone.

23. The method of any one of claims 18-22, wherein the population of cytokine-induced ML NK cells is pre-activated by exposure to IL-12, IL-18, and IL-15.

24. A method of producing a population of engineered cytokine-induced memory-like (ML) NK cell expressing a heterologous polypeptide, the method comprising:

(i) obtaining a population of primary human NK cells;

(ii) contacting the population of primary human NK cells with IL-12, IL-18, and IL-15 for a period of time sufficient to obtain a population of cytokine-induced ML NK cells expressing ASCT-2; and

(iii) contacting the population of (ii) with a pseudotyped lentiviral vector encoding the heterologous polypeptide under conditions to transduce the population of cytokine-induced ML NK cells;

wherein the pseudotyped lentiviral vector comprises a glycoprotein that binds to ASCT-2;

thereby producing a population of engineered cytokine-induced ML NK cells expressing the heterologous polypeptide.

25. The method of claim 24, wherein the glycoprotein is a retroviral glycoprotein.

26. The method of claim 25, wherein the retroviral glycoprotein is a baboon envelope (BaEV) glycoprotein.

27. The method of any one of claims 24-26, wherein the population of primary human NK cells is derived from iPSCs, cord blood, or PBMCs.

28. The method of any one of claims 24-27, wherein the population of primary human NK cells is autologous or allogeneic.

29. The method of any one of claim 24-28, wherein the period of time in step (ii) is about 12-16 hours, optionally about 14-16 hours, optionally about 16 hours.

30. The method of any one of claims 18-29, wherein the population of cytokine-induced ML NK cells

(i) produces increased IFNγ in the presence of one or more cytokines and/or tumor targets relative to a control human NK cell line;

(ii) has enhanced antibody-dependent cellular cytotoxicity relative to a control human NK cell line; and/or

(iii) has enhanced anti-tumor efficacy relative to a control human NK cell line,

optionally wherein the control human NK cell line is a human NK cell line activated in the presence of IL-15 alone.

31. The method of any one of claims 18-30, wherein expression of one or more of the following polypeptides is increased in the population of cytokine-induced NK cells relative to a control human NK cell line: CD94/NKG2A, NKp30, NKp44, NKG2D, and CD25.

32. The method of any one of claims 18-31, wherein one or more of the following polypeptides is relatively unchanged in the population of cytokine-induced NK cells relative to a control human NK cell line: KIR, CD57, NKG2C, DNAM-1 and CD137.

33. The method of any one of claims 18-32, wherein expression of CD16 and/or CD11b is decreased in the population of cytokine-induced ML NK cells relative to a control human NK cell line.

34. The method of any one of claims 18-33, wherein a plurality of the population of cytokine-induced ML NK cells are CD25+NKG2A+NKp30+NKp44+.

35. The method of any one of claims 18-34, wherein the pseudotyped lentiviral vector further encodes an IL-15 polypeptide, optionally a human IL-15 polypeptide.

36. The method of any one of claims 18-35, wherein the method further comprises isolating the population of cytokine-induced ML NK cells; and optionally, expanding the population of cells.

37. Use of the engineered cell or population of cells of any one of claims 1-14 or the pharmaceutical composition of claim 15, in the preparation of a medicament for treating cancer in a subject, wherein the cell surface of cells comprising the cancer displays an NPM1c neoepitope in complex with a class I major histocompatibility complex (MHC class I) protein.

38. The use of claim 37, wherein the cancer is AML.

39. The use of any one of claim 37 or 38, wherein the engineered cell or population of cells exhibits enhanced expansion following *in vivo* administration.